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Effects of Sodium Pentachlorophenate on Several Estuarine Animals Toxicity, Uptake, and Depuration

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Eastern oysters exposed to Na-PCP concentrations of 25.0 and 2.5 µg/ℓ accumulated the chemical in their tissues an average of 41 and 78 times, respectively. After Na-PCP delivery was discontinued, however, the oysters purged themselves of the pesticide within four days.

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Effects of Sodium Pentachlorophenate on Several Estuarine Animals: Toxicity, Uptake, and Depuration

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and LINDA F. FAAS

Abstract—Several estuarine animals were exposed to sodium pentachlorophenate (Na-PCP), in flow-through toxicity tests. The following are test animals and their 96-hr LC_{50} values: grass shrimp *(Palaemonetes pugio)*, > 515 μg/l; brown shrimp *(Penaeus aztecus)*, > 195 μg/l; longnose killifish *(Fundulus similis)*, > 306 μg/l; pinfish *(Lagodon rhomboides)*, 53.2 μg/l; and striped mullet *(Mugil cephalus)*, 112 μg/l. The 192-hr EC_{50} (effect measured was shell deposition) for the eastern oyster *(Crassostrea virginica)* was 76.5 μg/l.

Eastern oysters exposed to Na-PCP concentrations of 25.0 and 2.5 μg/l accumulated the chemical in their tissues an average of 41 and 78 times, respectively. After Na-PCP delivery was discontinued, however, the oysters purged themselves of the pesticide within four days.

Introduction

Pentachlorophenol (PCP) and sodium pentachlorophenate (Na-PCP) have been used as defoliants, herbicides, insecticides, molluscicides, wood preservatives and other applications for many years (Bevenue and Beckman, 1967). Over 23 million kg PCP were produced in the United States in 1971: approximately 18 million kg

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Mention of a commercial product does not constitute endorsement by the United States Environmental Protection Agency.

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(78%) was used in wood preservation; and nearly 17% (3.9 million kg) was manufactured as Na-PCP (von Rumker *et al.*, 1975).

PCP has been reported in numerous monitoring studies. The compound was found in drinking water (Abrams *et al.*, 1975) and sewage effluents (Abrams *et al.*, 1975; Benvenue *et al.*, 1972; Buhler *et al.*, 1973), as well as in aquatic biota (Zitko *et al.*, 1974). The occurrence of PCP in blood and urine of humans has been documented (Rivers, 1972).

PCP was implicated in severe fish kills in recent years in Europe (Holmberg *et al.*, 1972) and North America (Personal Communication, Dr. O.E. Langer, Environmental Protection Service, Dept. of Fisheries and the Environment, West Vancouver, B.C., Canada). To our knowledge, all reported cases were associated with the pulpwood industry.

Few data have been published on the toxic effects of PCP or Na-PCP on estuarine organisms. Testing PCP on the yellow eel *(Anguilla anguilla)* under static conditions, Boström and Johansson (1972) described its biochemical effects and Holmberg *et al.* (1972) described uptake and depuration.

In this paper, we report the results of: (1) the evaluation of toxicity of Na-PCP to brown shrimp *(Penaeus aztecus)*, grass shrimp *(Palaemonetes pugio)*, longnose killifish *(Fundulus similis)*, pinfish *(Lagodon rhomboides)*, and striped mullet *(Mugil cephalus)*; (2) the effect of Na-PCP on shell deposition of the eastern oyster *(Crassostrea virginica)*; (3) uptake of Na-PCP by animals that survived the 96-hr exposure; and (4) uptake and depuration of Na-PCP by eastern oysters in a 56-day study.

Test Animals

All test animals were collected near the Environmental Research Laboratory, Gulf Breeze, Florida. The shrimp and oysters were acclimated to laboratory conditions for at least 10 days prior to testing; fishes, at least 14 days. If mortality in a specific lot of animals exceeded 1% in the 48 hours immediately preceeding the test or if abnormal behavior was observed during acclimation, the entire lot was discarded. The shrimp and fishes were not fed during the 96-hour tests. The oysters used in the shell deposition study and in the 56-day uptake/depuration study obtained food (plankton) from the unfiltered seawater in which they were held.

96-hr Toxicity Tests

Test methods for shrimp and fishes were similar to those described by Lowe *et al.* (1972), except that seawater was filtered through swimming pool and sand filters and heated to at least 24 °C for all tests. Maximum temperature was 25.1 °C. Salinity was allowed to vary with that of Santa Rosa Sound (18 to 31 ‰). Test methods were also compatible with those of American Public Health Association (1975). Acute toxicity of sodium pentachlorophenate was determined by exposing 20 animals per aquarium (70-liter capacity) to each concentration for 96 hours.

Analytical grade Na-PCP was dissolved in deionized water and metered by pumps at 30 ml/hr into filtered seawater that entered each aquarium by siphons calibrated to deliver 31 l/hr. One control aquarium received the same quantity of seawater and no Na-PCP. Mortality was recorded daily and dead animals were removed when discovered. At termination, surviving brown shrimp, grass shrimp, longnose killifish, and mullet were analyzed chemically for PCP residue content.

The eastern oyster *(Crassostrea virginica)* shell deposition study was conducted in unfiltered seawater at ambient temperature using the procedures of Butler (1965), except that the test was extended an additional 96 hours (192 hours total exposure) to allow for reduced shell deposition caused by exceptionally low seawater temperatures (temperature of seawater ranged from 7.3 to 8.3°C; salinity, from 18.6 to 23.2 ‰). Shell deposition was measured to the nearest mm at the end of the exposure.

Bioconcentration of Na-PCP was studied by exposing 75 oysters (52 mm to 108 mm, umbo to distal valve height, $\bar{x}$ = 73 mm) to Na-PCP concentrations of 5.0 and 50.0 µg/l (average measured PCP concentrations: 2.5 and 25 µg/l, respectively) in flowing seawater. Stock solutions of Na-PCP, made by mixing the compound with appropriate volumes of deionized water, were delivered to each aquarium by syringe pumps. Unfiltered ambient seawater (salinity 23 to 30.5 ‰, $\bar{x}$ = 26.6 ‰; temperature 23.2 to 25.7°C, $\bar{x}$ = 24.2°C) was delivered to the two experimental and one control aquaria (165 l) at the rate of 440 l/hr. Three oysters from each aquarium were sampled twice each week to determine PCP in edible oyster tissues. The 28-day exposure to Na-PCP was followed by a depuration period in which the oysters were held in PCP-free seawater. The depuration period lasted until no PCP (< 0.01 µg/g) was detected in oyster samples from all aquaria in two successive sampling periods.

Chemical Analysis of PCP

One to eight grams of tissues were weighed into 150 mm (O.D.) screw-top test tubes and extracted four times with 5 ml portions of acetonitrile for 30 seconds using a model PT 10-ST Willems Polytron (Brinkman Instruments, Westbury, New York). The test tube was centrifuged after each extraction and the acetonitrile transferred to a 100-ml graduated cylinder with ground glass stopper. To the combined extracts were added 70 ml of 2.0% aqueous sodium sulfate; 4 drops of 6N hydrochloric acid were added to convert the Na-PCP to PCP for measurement. (The aqueous sodium sulfate solution was prepared with distilled water that had been acidified and extracted with hexane to remove interfering impurities). This solution was then extracted with one 10-ml portion of hexane, followed by two 5-ml portions. The graduated cylinder was stoppered and shaken for one minute, the solvent phases allowed to separate, and the upper hexane layer pipetted into a 25-ml Kuderna-Danish concentrator tube. The combined extracts were then concentrated to 0.5 ml by evaporation with a gentle stream of nitrogen in a water bath maintained at a temperature less than 50°C. The PCP-ethyl ether derivative was formed by addition of 10 to 15 drops of diazoethane that had been previously

generated and stored at -20°C (Stanley, 1966). Gloves and a high-draft hood were used in handling diazoethane, a toxic, potentially explosive carcinogen. This mixture was allowed to stand at room temperature for 20 minutes, and the excess diazoethane was evaporated with a gentle stream of nitrogen. The concentrate was quantitatively transferred with three 0.5 ml portions of hexane to 9-mm Chromoflex column (Kontes Glass Company, Vineland, N.J.) containing 3.0 g Florisil topped with 2.0 g anhydrous sodium sulfate that had been washed previously with 10 ml hexane. PCP-ethyl ether was eluted from the column with 20 ml of 5% diethyl ether/hexane solution and the volume adjusted to the appropriate level for analysis by electron-capture gas chromatography.

Water samples were analyzed in 100-ml aliquots, adjusted to a pH < 2 with 6N HCl. Each sample was extracted twice with 50 ml 1:1 diethyl ether/petroleum ether with a 250 ml separatory funnel. The extract, collected in a 25-ml Kuderna-Danish concentrator tube with 100-ml Kuderna-Danish evaporator, was concentrated to 5 ml on a steam table. The concentrator tube was transferred to a nitrogen evaporator, concentrated to 0.5 ml, derivatized with diazoethane and analyzed as for tissue samples, without the Florisil cleanup.

Determinations were performed on Hewlett - Packard Model 5730A electron capture gas chromatographs equipped with 182 cm x 2 mm (I.D.) glass columns packed with 2% SP2100 on 100/120 mesh Supelcoport and 5% QF-1 on 80/100 mesh Gas-Chrom Q. The operating parameters were: oven temperature, 200°C; injector temperature, 200°C; detector temperature, 300°C; carrier gas 10% methane/argon flowing at 25 ml/minute.

Residue concentrations are reported as pentachlorophenol, calculated on a wet-weight basis without a correction factor for percentage recovery. To evaluate the integrity of the results, all samples were fortified with an internal standard (O, P'DDE) prior to analysis. Average recovery rates of PCP and O, P'DDE from fortified tissue and water samples were greater than 90%. A new PCP standard was prepared and a glassware blank run with each set of samples. Also, all glassware was previously washed with 1N KOH solution and rinsed with distilled water, followed by nanograde acetone. These precautions were necessary to insure that there was no interference from impurities.

Statistical Methods

Shrimp and fish mortality data were analyzed by the probit analysis method of Finney (1971) to determine LC_{50} values and their 95% confidence intervals. Oyster shell deposition data were analyzed by linear regression with probit transformation to determine an EC_{50} (the concentration of PCP effective in reducing shell deposition of exposed oysters to 50% of that of control oysters) and 95% confidence intervals.

TABLE 1. Acute Toxicity of Sodium Pentachlorophenate (Na-PCP) to Several Estuarine Organisms

Species	Size[a] (x̄, mm)	96-hour EC_{50}[b], µg/l		Temp. (x̄, °C)	Salinity (x̄, ‰)
		Nominal	Measured		
Crassostrea virginica	45	104.0 (54-158)[c]	76.5 (37-116)	8.4	20.3
Penaeus aztecus	66	> 320.0	> 195.0	25.0	26.5
Palaemonetes pugio	18	> 560.0	> 515.0	24.8	24.3
Fundulus similis	42	> 560.0	> 306.0	24.4	22.9
Lagodon rhomboides	80	107.6 (93.7 - 122.0)	53.2 (42.4 - 65.4)	25.0	20.8
Mugil cephalus	58	221.6 (92.3 - 489.6)	112.1 (44.0 - 210.4)	24.7	25.5

[a]Size is height (umbo - distal valve edge) for oysters; rostrum - telson length for shrimp; standard length for fishes.
[b]Effect measured is shell deposition for oysters and mortality for shrimp and fishes.
[c]The 95% confidence intervals are in parenthesis.

Results of 96-hr Tests

Sodium pentachlorophenate was acutely toxic to oysters exposed for 192 hours (Table 1). The 192-hr EC_{50} was 76.5 µg/l. The EC_{50} value (measured) at the end of 96 hours approximately equalled that of the 192-hr EC_{50}, but due to the cold seawater temperature, control oysters deposited an average of only 1 mm shell/oyster. Therefore, we exposed the oysters for an additional 96 hours.

Exposure of brown shrimp to Na-PCP concentrations as high as 195 µg/l and the grass shrimp to concentrations up to 515 µg/l did not lead to significant mortality, or bioconcentration of the compound (Tables 1 and 2). Goodnight (1942) exposed various invertebrate species (including crustaceans) to 5 mg/l Na-PCP in static tests and reported that all but bloodworms were extremely resistant to the pesticide. Therefore, many invertebrate species appear to be relatively insensitive to Na-PCP compared to fishes.

Sodium pentachlorophenate at the concentrations tested, was acutely toxic to striped mullet and pinfish but not to longnose killifish (Table 1). Pinfish were the most sensitive species tested, the 96-hr LC_{50} being 53.2 µg/l. In comparison, Borthwick and Schimmel (this Symposium) reported a 96-hr LC_{50} of 38 µg/l for larval pinfish (< 48 hrs post-hatch) exposed to Na-PCP in a static test.

The lesser sensitivity of longnose killifish compared to pinfish in this test is similar to that shown by Schimmel *et al.* (1977a) with the insecticide toxaphene.

TABLE 2. Uptake of Sodium Pentachlorophenate (Na-PCP) by Several Estuarine Organisms

Species	Water conc. Measured (µg/l)	Whole-body residue (µg/kg wet wt)	Bioconc. factor
Penaeus aztecus	ND[a]	ND[a]	–
	14.0	10	–
	32.0	12	0.37[b]
	49.0	22	0.45
	92.0	11	0.12
	195.0	22	0.11
			$\bar{x}$ = 0.26
Palaemonetes pugio	ND	ND	–
	32.0	50	1.6
	54.0	100	1.9
	76.0	230	3.0
	249.0	430	1.7
	515.0	240	0.5
			$\bar{x}$ = 1.7
Fundulus similis	ND	ND	–
	36.0	1,100	31.0
	85.0	3,500	41.0
	287.9	3,300	12.0
	306.0	10,800	35.0
			$\bar{x}$ = 30.0
Mugil cephalus	ND	ND	–
	26.0	230	8.8
	46.0	290	6.3
	85.0	6,700	79.0
	157.0	8,800	56.0
	308.0	–	$\bar{x}$ = 38.0

[a]Control, non-detectable, = < 0.01 µg/l in water, < 10 µg/kg in tissue.
[b]Biconcentration factor = concentration of PCP in tissues divided by the concentration of PCP in the external medium.

The reason for this range of sensitivity is unclear, but other investigators have reported a similar phenomenon when comparing susceptibility of freshwater fishes from various taxonomic groups to organochlorine insecticides (Macek and McAllister, 1970).

Our data on the toxicity of Na-PCP to estuarine fishes compare favorably with those for freshwater fishes. In static tests, the 96-hr LC_{50} value for bluegills *(Lepomis macrochirus)* was 20 μg/l; for goldfish *(Carassius auratus)*, 50 μg/l (Inglis and Davis, 1972). The 96-hr LC_{50} for the sockeye salmon *(Oncorhynchus nerka)* exposed in flow-through toxicity tests was 63 μg/l (Webb and Brett, 1973). For brook trout *(Salvelinus fontinalis)*, fathead minnows *(Pimephales promelas)*, goldfish, and bluegills exposed to Na-PCP in acute flow-through toxicity tests, a total mortality of all species was reported to occur at concentrations < 1.0 mg/l (Cardwell *et al.*, 1976).

Tissues of surviving longnose killifish and striped mullet from the 96-hr toxicity tests were analyzed for PCP. Results indicated that PCP was bioconcentrated by the killifish to an average of 30 times the amount measured in the exposure water and by mullet, 38 times (Table 2). These bioconcentration factors are extremely low compared to the bioconcentration (up to 1000×) by goldfish exposed to media containing PCP (Kobayashi and Akitake, 1975). Whether these contrasting results are attributable to possible differences in the rate of uptake of PCP among the fishes studied, or due to differences in the experimental procedures, remains uncertain.

The bioconcentration factors we observed were also extremely low when compared with many other organochlorine insecticides. For example, in other 96-hr studies, the average bioconcentration factor for pinfish exposed to toxaphene was 3,850 (Schimmel *et al.*, 1977a); for chlordane, 6,228 (Parrish, *et al.*, 1976) and for benzenehexachloride (BHC), 482 (Schimmel *et al.*, 1977b).

Uptake and Depuration of PCP by Oysters

Eastern oysters exposed to measured PCP concentrations of 25.0 and 2.5 μg/l for 28 days accumulated the chemical in their tissues an average of 41 and 78 times, respectively (Figure 1). Pentachlorophenol reached an apparent equilibrium in oysters within the first four days in both exposure concentrations and remained relatively constant throughout the uptake portion of the study. The mean concentration of PCP in oysters from the 25 μg/l aquarium was 1060 μg/kg; for the 2.5 μg/l aquarium, was 180 μg/kg. After the PCP delivery was discontinued, however, the oysters purged themselves of the pesticide within four days.

In summary, Na-PCP reduced shell deposition of eastern oysters at concentrations ≥ 34 μg/l during a 192-hr exposure. Concentrations of the pesticide as high as 195 and 515 μg/l, however, were neither toxic to brown shrimp and grass shrimp, respectively in 96-hr exposures, nor did the animals bioconcentrate the chemical. Pinfish were the most sensitive of all species tested (96-hr LC_{50} = 53 μg/l).

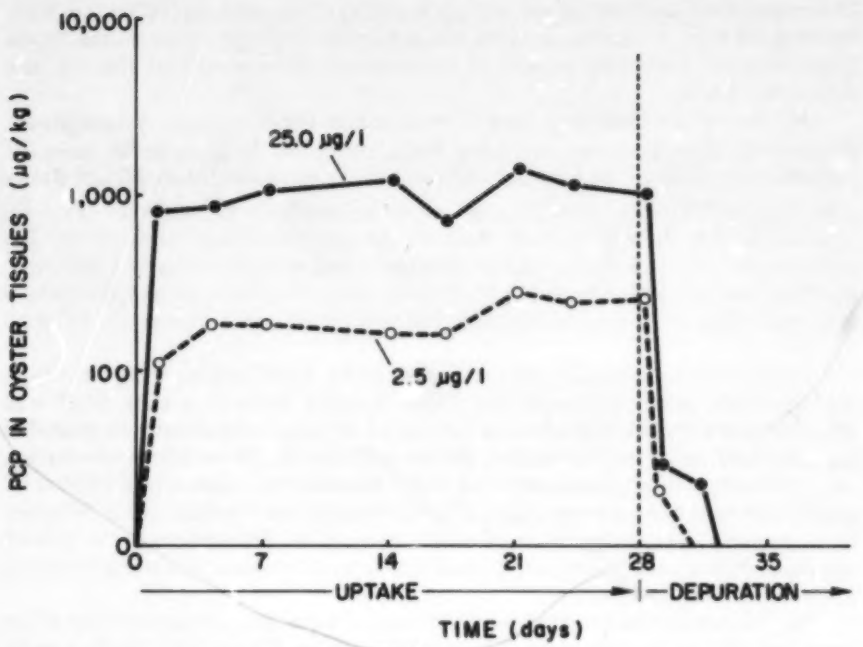

Figure 1. Uptake of pentachlorophenol (PCP) by eastern oysters *(Crassostrea virginica)* exposed 28 days, then allowed to depurate in PCP-free seawater.

Oysters bioconcentrated PCP 41 to 78 times the amount measured in water and when held in PCP-free seawater, depurated the chemical to nondetectable concentrations in four days. Compared with other organochlorine insecticides (such as toxaphene, chlordane and BHC) tested on estuarine animals, Na-PCP is relatively less toxic and is bioconcentrated to a lesser extent.

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